The Pharmacology of Indaconitine and Bikhaconitine.

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The present paper deals with the physiological action of two new "aconitines," which have been isolated at the Imperial Institute from two varieties of Indian aconite. One is an alkaloid, which has been named indaconitine. It was found in the roots of the Indian aconite, called by Bruhl, *Aconitum napellus*, var. *hians*, since identified by Stapf as a new species, which has received the name of *Aconitum chasmanthum*. *Aconitum chasmanthum* being abundant in India, the highly toxic alkaloid derived from this plant has been called "indaconitine," a name appropriate to the properties of this alkaloid, which closely resemble those of aconitine derived from the common European aconite, *Aconitum napellus*.

The other alkaloid has been named "bikhaconitine," being derived from one of the highly poisonous forms of aconite known in India under the vernacular name of "Bikh." This aconite was named by Bruhl, *Aconitum ferox*, var. *spicatum*, but has been re-named *Aconitum spicatum* by Stapf, who regards it as a distinct species.

The chemistry of these two alkaloids, which has been fully worked out at the Imperial Institute, will be described by one of us in a separate communication. It will be sufficient to allude here to the leading chemical characters of the two substances.

Indaconitine differs only slightly from aconitine in its composition and properties, but in several respects these differences are well marked. Indaconitine crystallises well, but its usual crystalline habit is distinct from that of aconitine, although the crystallographic characters of the two substances are very similar, and they may prove to be isomorphous. Most of the salts crystallise readily, and the hydrobromide has been employed for the study of the physiological action of the alkaloid.

Like aconitine, indaconitine undergoes hydrolysis in two stages. Partial hydrolysis leads to the separation of a molecule of acetic acid, and the formation of a base which has been named benzoyl-pseudaconine. This substance on further hydrolysis furnishes one molecule of benzoic acid, and a base which proves to be identical with pseudaconine, the ultimate hydrolytic product of pseudaconitine derived from forms of *Aconitum ferox*, the chemistry and pharmacology of which have been described in previous

papers. Indaconitine therefore contains the acetyl and benzoyl groups present in aconitine of European origin, associated with the basic nucleus of the Indian pseudaconitine. Its pharmacology as described in the present paper corresponds with its chemical relation to these two alkaloids.

Bikhaconitine closely resembles pseudaconitine, but is chemically distinct from it. The alkaloid and its salts crystallise well. Similarly its derivatives somewhat resemble those of pseudaconitine, but are distinct substances. On partial hydrolysis bikhaconitine furnishes one molecule of acetic acid and veratryl-bikhaconine, which on further hydrolysis furnishes one molecule of veratric acid and bikhaconine. Bikhaconitine is therefore, chemically, the analogue of pseudaconitine, and is also its pharmacological congener. It is only slightly inferior in toxic power to pseudaconitine, which is the most poisonous aconitine yet examined.

The examination of the physiological action of indaconitine and bikhaconitine has been carried out on parallel lines with that of the alkaloids aconitine, pseudaconitine, and japaconitine, which have been previously discussed.* In each case the hydrobromide was the salt employed. It is proposed here to give very briefly, often in form of synopsis, the main results arrived at, including the dosage which is associated with a lethal action.

Indaconitine will be first considered.

Indaconitine.

Effect upon Blood-Pressure, Pulse, and Respiration of Anæsthetised Animals (Cats and Rabbits).

There is a striking similarity with the effects produced by parallel doses of aconitine (from *A. napellus*). The phases of slowing of the pulse (with or without a slight anterior acceleration) marked quickening, and subsequent arhythmia, due to incoordinate action of auricles and ventricles, are all present, whilst similar changes in the blood-pressure, culminating in the rapid and extensive fluctuations so characteristic of aconitine, are occasioned also by indaconitine. Under ether there is little (1/6 to 1/5) or none of the primary acceleration of respiration which usually occurs in non-etherised animals (see later), but a gradual slowing, and eventually a failure of effective respiratory movements is witnessed, this condition speedily leading to a fatal issue if vigorous artificial respiration is not employed.

Vagus section and stimulation have a similar result during the toxic conditions occasioned by aconitine and indaconitine respectively, the acceleration of cardiac action, and the more effective systole due to a closer

* See 'Phil. Trans.,' B, vol. 190, 1898, and abstract in 'Proceedings,' vol. 62; 'Phil. Trans.,' B, vol. 195, 1903, and abstract in 'Proceedings,' vol. 68.

sequence of ventricular upon auricular action, with a resulting rise in the pressure being observed on stimulating during the stage of cardiac irregularity. The proportion of indaconitine capable of producing such results was 0·0011 gramme per kilogramme and upwards, administered hypodermically in a single dose. When one-half of the lethal proportion (per kilogramme) was repeated at intervals of 45′, the third dose was followed in rabbits by great irregularity of the pulse, wide fluctuations in blood-pressure, and rapid decline in respiratory frequency. The long inspiratory pause, with the forced effort at its commencement, became more marked as the toxic action progressed. This proportionate dose repeated twice at intervals of 45′ may prove lethal, though this is an exceptional occurrence.

No estimation was made of the lethal dose of indaconitine for non-anæsthetised cats, but taking the proportion as 0·00012 which is applicable to rabbits, it was found that half this dose, administered hypodermically and repeated every 45′, was followed by death in etherised cats about 70′ after the third injection. The effects were thus developed:—

After First Injection.—Acceleration (transitory) and slowing of the pulse. Moderate fall of arterial pressure. Slight acceleration followed by slowing of the respiration.

After Second Injection.—Continued slowing of the pulse. Thereafter acceleration. Blood-pressure fairly steady. Further slowing of the respiration. Usual results of peripheral vagus and splanchnic stimulation.

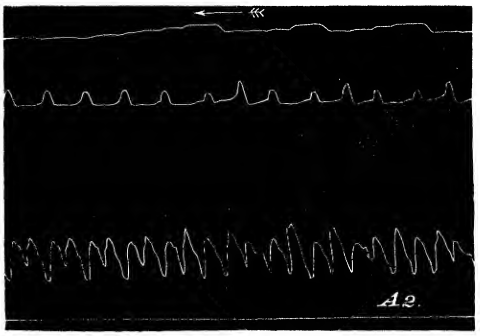
After Third Injection.—Irregularity of the pulse increasing as time progressed. The heart greatly accelerated, attaining 200 per minute. Immediately before death, and when the blood-pressure amounted to 28 mm. of mercury, the rhythm became regular. In the earlier part of this period, vagus stimulation co-ordinated the action of the auricles and ventricles, temporarily raising the blood pressure, but this effect was lost later. Splanchnic reaction was never entirely abolished. The respiration, at first very slow but effective, became weaker and was suspended. Artificial respiration served to prolong life for over 20′. The tracings (A_{1-3}) are taken from an experiment in which registration of carotid pressure and contraction of the left auricle and ventricle were taken simultaneously.

A_1. Before injection.

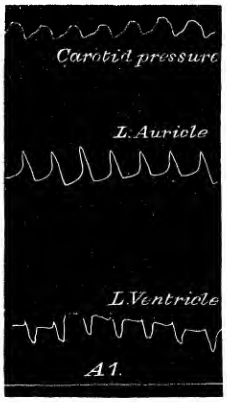
A_2. 50′ after the third injuction of 0·5 lethal dose of indaconitine. (The injections occur every 45′.)

A_3. 70′ after the third injection, and 5′ before death.

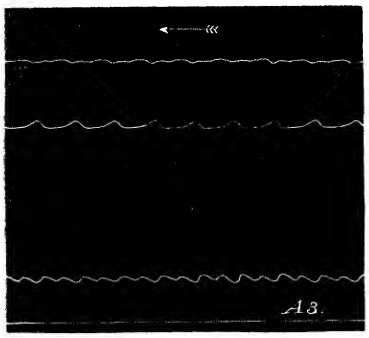
50 mins. after third injection of one-half of lethal dose of indaconitine.

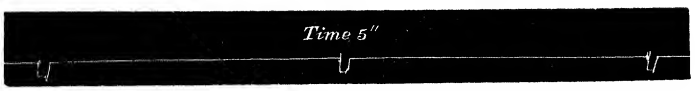


Before injection.



70 mins. after. 5 mins. before ex. leth.



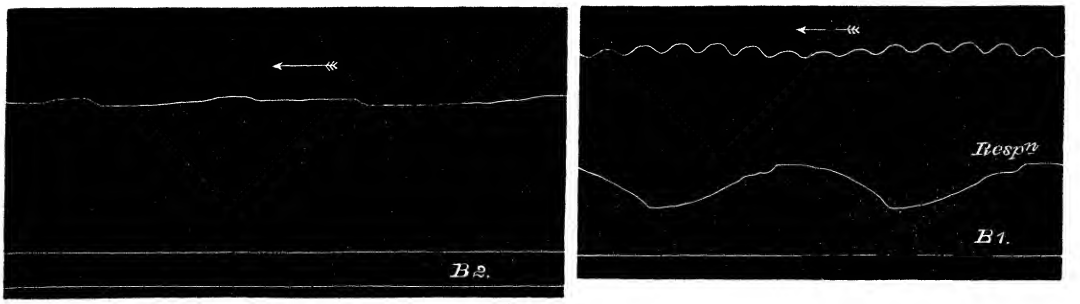


Antagonism of Atropine.

The antagonism of atropine and indaconitine is of the same character as that which has been described as existing between atropine and aconitine. Not only is the irregularity of the pulse reduced (the heart being slowed and the sequence of ventricular action restored), but the blood-pressure is increased and steadied, whilst the respiration, which may have been abolished as an effective function, is rapidly reinstated, the original speed being attained or approached.

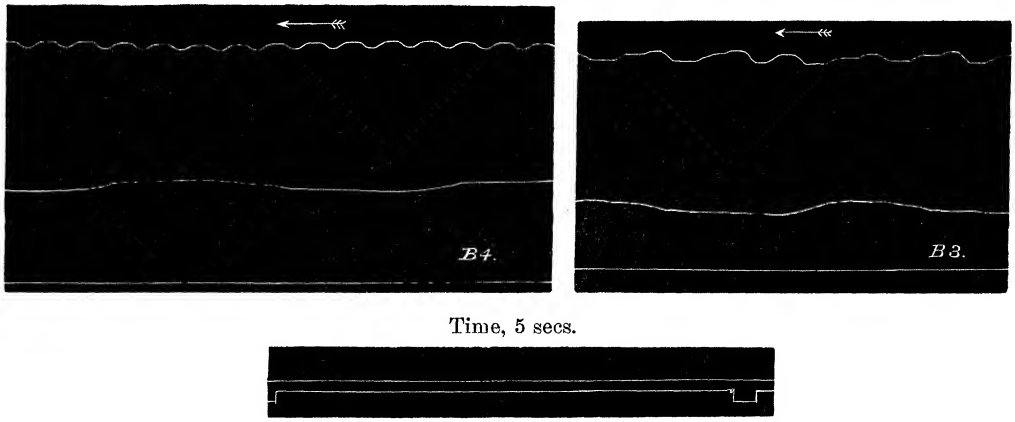
In the following experiment (B_1), injections were made by the femoral vein of an ætherised cat of repeated small doses of indaconitine. In 50′ a proportion of 0·00016 gramme per kilogramme had been totalled; irregularity of the heart was present, and respiration was represented only by

After 0·00016 per kilogramme indaconitine. Before injection.



an occasional and inefficient movement, rendering articial insufflation necessary. In the following 25′ an additional injection of 0·000022 indaconitine was made (B_2). Five minutes later the heart was beating very rapidly (over 300) and irregularly (36 impulses per minute in the carotid), whilst the blood-pressure was falling so fast that a lethal issue was obviously impending. Injection of 0·006 gramme of atropine sulphate was now made by the femoral vein, and the serious symptoms soon began to abate, so that 20′ later carotid pressure was 56 mm., carotid impulses (though very irregular) 77, and respirations 15 per minute. During the succeeding 25′ further injections of atropine sulphate amounting to 0·0089 gramme were made; the pulse remained irregular, and often bigeminal in character; pressure 65 mm., and respirations 50 per minute (B_3). A further injection of 0·004 gramme of atropine restored the regular beat of the pulse to 154 per minute, the pressure rising

Complete antagonism by atropine. Partial antagonism by atropine.



to 96 (B_4). Thus the effects of indaconitine, amounting to 0·000182 gramme of the alkaloidal salt per kilogramme, were fully antagonised by 0·018 gramme

of atropine sulphate (0·01 per kilogramme) in the course of 65′. The pulse and pressure having remained steady for a period of 40′, the interrupted injections of indaconitine were resumed, when it required no less than 0·00027 gramme per kilogramme (administered in the course of 50′) to reproduce a distinct aconitine effect. It is probable, therefore, that atropine had been used in excess of the antidotal equivalent required by the original indaconitine injection.

Action of Indaconitine on Rabbits.

A single hypodermic injection of 0·00008 gramme per kilogramme of indaconitine produced acceleration of the respiration, then slowing of respiratory rhythm to one-half or thereabouts of the original rate, salivation, dilatation of the pupil and a fall of rectal temperature amounting to about 1·5° C. These effects were transitory, as salivation lasted for less than 30′, whilst respiration had returned to, or nearly, to the original, and the temperature was beginning to recover 90′ after injection had been made.

0·00011.—Sharp acceleration of respiration succeeded by slowing to 14 or 16 per minute. Salivation ushered in by active chewing and retching movements. Early pupillary dilatation. Marked dyspnœa, with some retraction of thorax on inspiration; occasional dyspnœal spasm. Moist large crepitations in air passages. Much paresis of limbs. The total fall of temperature amounted to between 2 and 2·5° C. The symptoms were abating in 110′.

0·00012 per kilogramme.—Transitory respiratory acceleration followed by great slowing. Salivation and dilatation of the pupil in 15′. Considerable paresis and respiratory spasm. Death occurs in 50′ to 60′.

Lethal Dose.—Although an occasional lethal result may follow doses of the indaconitine salt proportional to 0·0001 per kilogramme, it is not until one-fifth more is given that the result becomes almost invariable. The lethal relationship stands accurately at 0·00012 gramme per kilogramme—only once has this dose been exceeded, with a different issue.

Repeated Administration of Indaconitine.—On Temperature and Respiration.

Indaconitine causes an acceleration of respiration immediately after injection of doses of one-third of the lethal proportion and upwards, especially if the original respiratory rhythm had been less than 60 per minute. But if the original rate is twice as rapid, then there may be an absence of acceleration. The tendency to acceleration becomes less as the proportion of aconitine becomes smaller.

In all cases, whether there be a primary rise or not (after one-third of the lethal and upwards), a fall in the respiratory rate follows injections of indaconitine. This may amount to one-half of the original, after a dose of one-

half of the lethal, or a reduction to from one-quarter to one-fifth after five-sixths of the lethal. In the latter case the movements are distinctly dyspnœal, retraction of the chest wall, fixation of the thorax and relative prolongation of the inspiration being present. The acceleration which sooner or later succeeds to this slowing (the dose being sub-lethal) is often to a rapidity of rhythm much beyond the original. This is more particularly the case when doses of about half lethal are given than when the proportion is larger. The acceleration occurs earlier in the course of readministrations of indaconitine than after equivalent doses of bikhaconitine.

Repeated Administration of Indaconitine at Stated Intervals to Rabbits.

Effect on Temperature and Respiration.

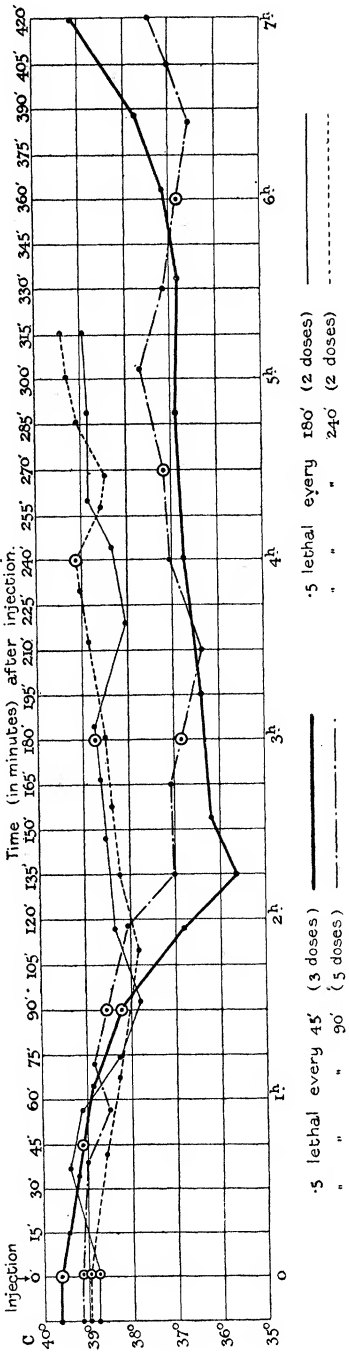
Fraction of lethal proportion per kilo. body weight.	When repeated.	How often.	Total proportion to the lethal.	Greatest fall of temperature (C.).	Subsequent course of temperature.	Note on respiration.
	mins.			C.		
$\frac{1}{6}$	45	7	$1\frac{1}{6}$	1·0	Greatest reduction after second injection. Thereafter broken rise.	
„	60	6	1·0	0·9	Greatest after second injection.	
$\frac{1}{4}$	45	8	2·0	2·3	Greatest after fifth injection, when broken rise ensues.	Eventual acceleration of respiration, more rapid than in case of bikhaconitine.
„	60	6	1·5	1·2		
$\frac{1}{2}$* (Diag. A)	45	3	1·5	3·8	Greatest after third injection. In 420′ is still −1° C.	Respiration reduced to 13 per minute. Rises with the temperature.
„ (Diag. A)	90	3	1·5	2·8	Greatest after second injection.	
„	120	3	1·5	1·6	Greatest after first injection.	
„ (Diag. A)	180	2	1·0	1·0	Rise of 0·7° C. Fall greatest after first injection.	
„ (Diag. A)	240	2	1·0	1·2	Greatest after first injection.	

* Such administrations (0·5 lethal twice repeated at intervals of 45′) were made in three experiments, in one of these with lethal result (see p. 470).

Toxic Action of Indaconitine in Progressive Doses towards Frogs.

0·0006 gramme per kilogramme weight of *R. temporaria*. Some excitement (soon subsiding) after injection. No marked impairment of reflexes. Acceleration of respiration succeeded by slowing.

DIAGRAM A.



Effect of Indaconitine on the Internal Temperature of Rabbits. Administrations are indicated by the mark ⊙.

0·0009.—Considerable excitement with irregularity of reflexes in limbs and inability to assume ventral position when placed on dorsum. The lungs are in the main widely inflated; respiratory movements occasional.

0·0012.—After initial excitement with frothing there is slowing and suspension of respiration—gaping. Reflexes much impaired and very uncertain. Prolonged inability to get off dorsum. Recovery not complete for five days.

0·0013.—This is a hyperlethal proportion. After excitement there is rapid failure of voluntary movement. Reflexes are diminished and uncertain in half an hour after injection. Respiration, after brief acceleration, is slowed and abolished. Heart's action becomes incoordinate. If death occurs (from cardiac failure) in five to six hours, some reflex may occasionally be elicited even when circulation has ceased, but if the lethal issue is postponed till some hours later, the limb reflexes disappear first, the body being limp and flaccid. The ventricle shows characteristic sacculations containing blood. The gastrocnemius is excitable to direct stimulation, usually the response to indirect stimulation is impaired.

The *lethal dose* of indaconitine varies in different seasons of the year. For *R. temporaria* in June and July a proportion of 0·00120 gramme per kilogramme was lethal on several occasions, and the proportion of 0·00125, which may be regarded as the lethal, was only twice exceeded (0·0014 and 0·00145) with subsequent recovery.

Action of Indaconitine upon the Cardiac Rhythm.—Lethal and slightly hyperlethal doses of indaconitine cause acceleration of the heart of brainless frogs, amounting to from four to eight beats per minute. The acceleration may be progressive after injection, or may be preceded by slight slowing (two to three per minute). After a duration, which varies largely according to the dose, acceleration passes into irregular rhythm with incoordinate action, the ventricle being chiefly affected. It is after this condition has lasted for a time that the characteristic "pouching" of the ventricle is produced by indaconitine, as it is by all the aconitines hitherto examined in this research. Subsequently, after lethal doses, a progressive slowing and enfeeblement of the ventricular beat occurs, until spontaneous action ceases. At this time the auricles are beating with some degree of regularity at a rate of 10 to 16 per minute. Local application of atropine restores spontaneous contraction of the ventricle to some extent.

Perfusion of Frog's Heart.—The main results of the perfusion of the separated ventricle by weak doses of indaconitine, are to increase excitability, accelerate the rhythm, and favour the occurrence of group beating. Large doses—0·0004 to 0·0006—produce rapid deterioration in the strength and

duration of the systole, but as an early effect, the excitability was increased, spontaneous contractions often appearing after perfusion by indaconitine, whilst no such effect had followed from the use of the simple nutrient fluid. The weakened and rapid beat of indaconitine is rendered slower and stronger when atropine solution is substituted.

Action on Respiration.—After temporary acceleration, slowing of the respiratory movements accompanied by periodical wide inflation of the lungs, follow medium doses of indaconitine, the flank movements being more obviously slowed than those of the hyoid. A proportion of 0·0006 to 0·0008 per kilogramme, whilst having such an effect, does not arrest the respiration, but with a proportion of, and above 0·001 per kilogramme, entire suspension of respiration may ensue, so that when the lethal dose is nearly reached, an absence of respiratory movement (obvious and registrable) may be observed for 24 hours or more. This condition may be coincident with some retention of reflex in the limbs. Before the animal resumes its power of regaining the ventral position when placed on the back, there is some return of respiratory movement.

Hyperlethal doses up to 0·0025 gramme per kilogramme will abolish respiration in from 30′ onward according to their extent. The last evidence of some activity in the respiratory mechanism is elicited by cutaneous stimulation, and especially by placing the frog on the dorsum.

Action on Reflex.—Sensory depression at the periphery is somewhat less powerful in the reflex (brainless) frog, under the influence of indaconitine, than it is under aconitine when equal proportions are given. Otherwise the general character of the effect is similar.

Reaction of Nerve Muscle Preparation.—These experiments were conducted upon brainless frogs, in some, vascular ligature being previously applied to one leg. The dose used was in proportion to the total weight of the frog, irrespective of the parts excluded from the circulation.

Experiments.—*Rana esc.* with brain destroyed received 0·00200 gramme of indaconitine per kilogramme. In five hours, the circulation having completely ceased 90′ previously, the nerve stimulated at 43 cm., the muscle at 16, yielded contraction of the muscle. Maximal stimulation, both direct and indirect, gave a series of fair contractions.

R. temporaria (brain destroyed, left vascular ligature) received the large proportion of 0·024 per kilogramme. In 80′ (heart arrested) minimal excitability, nerve 43, muscle 23 cm.

If after poisoning by a large dose of the alkaloid, the preparation of the nerve be delayed for 24 hours, a great reduction in excitability is witnessed, and the resulting contractions to stimulation are very much diminished in

force. Though this statement applies in degree to direct as well as to indirect stimulation, the failure of the nervous intramuscular structures is relatively greater than that of the muscle fibre.

After poisoning by as much as 0·007 to 0·008 gramme per kilogramme, entire absence of all response, even to direct stimulation applied 24 hours later, has been witnessed. Such a result is however exceptional.

In order to test as closely as possible the actual and relative effects of indaconitine and spicaconitine upon nerve and muscle, immersion experiments were employed. Ringer's perfusion solution was the menstruum used, this being well suited to the preservation of activity in nervous as well as in muscular tissue, as demonstrated by the fact that in control experiments in which 20 c.c. of this solution was used for immersing the muscle nerve preparation (the foot being retained as described in 'Phil. Trans.,' B, vol. 195, p. 66), the nerve preserved its excitability for from 45 to 50, and the muscle for 75 to 86 hours. Accurately measured amounts of indaconitine and bikhaconitine solutions were tested on companion nerve-muscle preparations obtained from the same animal, or else one of the preparations was exposed to an aconitine solution whilst the other was used as a control. In all cases the minimal excitability was determined from time to time with as little repetition of stimulation as possible.

With solutions having a proportion of alkaloidal salt of 1/5000 to 1/25000, the effects of indaconitine and bikhaconitine are fairly parallel towards nervous as well as muscular tissue; but with solutions of 1/50000 and up to 1/500000, indaconitine shows a somewhat greater activity relatively towards both structures, though the difference is more marked towards nervous tissue. The most dilute solution of indaconitine which proved directly active upon muscular tissue was 1/800000.

Solutions of 1/1000000 indaconitine (which do not affect muscular tissue) abolish the contractility of the specimen indirectly stimulated four to five hours before parallel solutions of bikhaconitine do so, and even with 1/2000000 the action of indaconitine usually preponderates, though but slightly. In a dilution of 1/2500000 bikhaconitine, the nerve muscle preparation frequently reacts as long as a control preparation to which no addition of an aconitine has been made. The excitability of intramuscular nerve structures is increased by both solutions when of medium attenuation, whilst the production by either of fibrillation is but seldom observed.

The resistance of the nerve muscle preparations in this series taken from frogs very recently obtained, was greater than that of those used in the immersion experiments made with japaconitine and pseudaconitine, upon animals which had been some time in the laboratory. It has not been

possible hitherto to institute a thorough comparison of the two series of aconitines under precisely similar conditions, but from the results of a few contrasted experiments, it seems likely that there is no large variation in the activity of the alkaloids under discussion from those previously examined towards muscular and intramuscular motor nervous tissue.

Bikhaconitine.

Action of Bikhaconitine upon Blood-pressure and Respiration of Anæsthetised Animals (Cats and Rabbits).

The general features of aconitine are reproduced by bikhaconitine with regard to the circulatory system, but the latter develops a stronger action upon respiration, which is slowed and altered in character. Though not so active in this respect as pseudaconitine, it is more so than the other aconitines examined in these researches, and it is to this that the greater toxicity of bikhaconitine is attributable.

In one experiment after the administration of three half lethal doses of bikhaconitine with intervals of 45′, a condition of "delirium cordis" was occasioned by strong vagus stimulation. Irregularity of the heart had already commenced and vagus stimulation (coil 10) had produced a rise of pressure with acceleration of the rhythm by 10 beats per minute, when after a pause of 15″ the secondary coil was approximated to 8 cm., with the above result. The auricle remained in a state of contraction but exhibiting rapid twitchings, the blood-pressure rose for 3″ and then fell rapidly, so that 5″ after the condition commenced the last of the irregular impulses had been recorded. The ventricles showed wild fluctuation apparently in every portion, which persisted for some time after all impulses had ceased in the carotid. No such result has been witnessed hitherto during the numerous experiments performed with the aconitines. Perhaps in this solitary instance it may have been due to the sudden exhaustion of the residue of co-ordinating action left in the vagus mechanism. The injection of atropine after the aconitines has never occasioned this phenomenon.

When the effect of bikhaconitine upon anæsthetised cats and rabbits is contrasted, it appears that the inhibitory action of the stimulated cardiac vagus is sooner abolished in the latter, but that at the time when this occurs section of the vagus still causes increased slowing of the respiration.

Action of Bikhaconitine on Rabbits.

A proportion of 0·00004 gramme per kilogramme (this is slightly less than one-half the lethal) causes acceleration followed by some degree of slowing of the respiration. Salivation rarely occurs. There is no paresis, but the

animal is quiet and does not feed for a time. The variation in rectal temperature is not more than 1° C., a slight rise may or may not precede the fall.

0·000065 gramme per kilogramme.—The respiratory changes noted above are accentuated. Free salivation accompanied by chewing and retching movements, some paresis, especially of the hind legs, pupillary dilatation, and decline of temperature by 1·5 to 2° are observed. Recovery takes place in two hours.

0·00008.—As the lethal proportion is approached all the symptoms are exaggerated. After transitory acceleration, there is respiratory slowing to one-quarter or one-fifth of the original. The respiration is for a time dyspnœal, the thorax expands but imperfectly, a prolonged pause in inspiration is usual. Respiration may become strident, and when this is the case dyspnœal spasms may occur. The pupils are dilated. Paresis is considerable, the body becoming limp, this condition extending in degree to the sphincter ani. The fall of temperature amounts from 2 to 3° C., and if the body is not kept covered or exposed to warmth, a reduction of many degrees more is probable and death may result. On one occasion even with such precautions death resulted after a dose of this proportion. Recoveries, however, occur up to 0·0000875 gramme per kilogramme.

0·00001.—This proportion is distinctly hyperlethal, death taking place in about 40′ and being primarily due to respiratory failure. It is preceded by dyspnœal convulsions.

The lethal dose has been determined at 0·0000875 gramme per kilogramme. On one occasion only has a slightly larger proportion been recovered from (0·00009).

From the tables of the effect of readministration of indaconitine and bikhaconitine respectively, as well as from experimental records which are not tabulated, the following points may be indicated:—

1. For both alkaloids there is a marked tolerance when the dose is one-sixth of the lethal administered every 45 or 60′, or even of one-quarter when repetition is not more frequent than hourly.

2. The greatest reduction after such administrations is after the second dose, and thereafter the temperature rises, though showing trifling checks when subsequent administrations are in progress.

3. After smaller, as well as after larger doses, it is peculiarly in those cases in which respiration remains depressed that the relatively late occurrence of the maximum fall of temperature is to be looked for. In fact it may be predicted that the temperature will continue to fall, or at least, fail to rise, so long as respiration remains in the region of 30 to 35 per minute.

Repeated Administration of Bikhaconitine at Stated Intervals to Rabbits. Effect on Temperature and Respiration.

Fraction of lethal proportion per kilo. body weight.	When repeated.	How often.	Total proportion to the lethal.	Greatest fall of temperature.	Subsequent course of temperature.	Note on respiration.
	mins.			°		
$\frac{1}{6}$	45	7	$1\frac{1}{6}$	1·1	Greatest reduction after second injection.	Respiration varies through 30 per 1′ only.
„	60	6	1·0	0·8	Greatest after second injection.	
$\frac{1}{4}$	45	8	2·0	3·5	Greatest after fifth injection.	Respiration remains slow, 34 to 48 per 1′ during prevalence of low temperature.
„	60	6	1·5	1·2		
$\frac{1}{2}$	45	2	1·0	3·2	Greatest after second. A third would have been fatal.	Active dyspnœa induced.
„	90	3	1·5	2·9	Greatest after second injection.	Respiration slowed by 30.
„	120	2	1·0	1·7	Greatest after second.	
„	180	2	1·0	1·7	Greatest after second. The two falls are equal in extent.	Respiration much slowed after each injection.
„	240	2	1·0	1·3	Greatest fall after first injection.	Falls to one-half pre-existing rhythm after each injection.

4. When such a proportion as one-quarter of the lethal is administered every 45′, or one-half of the lethal every 45, 60 or 90′, there is a decided summation of effect, and a further, often considerable reduction of temperature ensues upon readministration, so that in the former case the maximal fall does not occur until after the fifth administration.

When a third administration of the half lethal dose of indaconitine is made at intervals of 45′, a lethal issue may occur, though this is exceptional, but the third dose of bikhaconitine so administered is lethal.

5. As between bikhaconitine and indaconitine, it is further observable that the respiration accelerates less rapidly after the repetition of the former than when the latter is given, and to this may frequently be ascribed the greater accession to the fall of temperature after bikhaconitine. It appears that as a result of greater summation of effect produced by bikhaconitine, when doses larger than one-sixth of the lethal are administered, the action of the alkaloid becomes relatively greater than that of indaconitine. The toxicity of bikhaconitine is upon repetition of such doses, greater with regard to indaconitine than the relationship of the unit lethal dose, viz., 0·0000875 gramme to 0·00012 per kilogramme, would indicate.

6. Up to 180′ there is usually summation of bikhaconitine effect after a 0·5 lethal dose, whereas summation after indaconitine for a parallel proportion is rarely witnessed after a longer interval than two hours.

Action of Bikhaconitine towards Frogs (R. temporaria).

(A Synopsis of Experiments is appended.)

0·0009 gramme per kilogramme.—Excitement, then voluntary movement reduced, limb reflexes often uncertain, respiration slowed, ultimately suspended.

0·0012.—Excitement, with frothing on the body. After transitory acceleration, slowing and arrest of respiration, all voluntary movement disappearing, gaping movements, great impairment of reflexes, lasting 4 to 5 days, during which period there is inability to get off the dorsal position.

0·0013.—As above; but cardiac irregularity develops, with ultimate failure of effective systole, the circulation in the web becoming partial, feeble, and intermittent until it ceases altogether. Death takes place in 12 to 14 hours after injection.

Lethal Dose.—In June and July an occasional lethal effect followed a proportion of 0·001 gramme and upwards, until on reaching 0·00125 gramme per kilogramme recoveries were very exceptional. The last may be accepted as the lethal proportion.

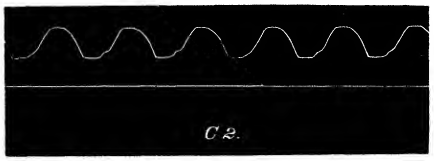
Whilst the action of bikhaconitine is both qualitatively and quantitatively closely similar to that of indaconitine on the heart, the effect of bikhaconitine is greater than that of indaconitine on the frog's respiration.

Action on Respiration.—Suspension of respiration is not essentially inimical to the continuance of life in frogs, and therefore, though bikhaconitine causes an arrest of visible or registrable movement in smaller proportion than indaconitine would, the lethal proportion is practically the same for the two alkaloids. Whilst proportions of 0·0008 to 0·0009 of bikhaconitine may suspend respiratory movement for from $1\frac{1}{2}$ to $2\frac{1}{2}$ hours, doses approaching the lethal prolong the period of inactivity to 36 hours or more.

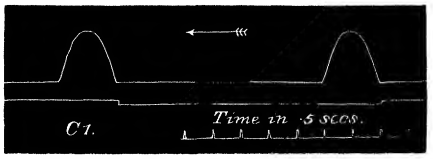
Bikhaconitine upon the Cardiac Rhythm of Frogs.—The effects of this alkaloid are essentially those of indaconitine, as are also the modifications produced in the reaction of the heart to vagus stimulation.

Perfusion of the separated organ has also parallel results. Fig. C_2, C_3 shows the increased excitability, accelerated rhythm, and failing systole of the ventricle under perfusion of relatively powerful solutions of the alkaloid (0·00048 and 0·000549). The two contractions in C_1 are elicited by stimulation before circulation of bikhaconitine.

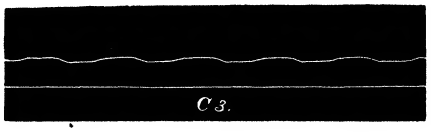
After 0·00048 gramme bikhaconitine.



Perfusion of ventricle with normal solution.



After 0·00064 bikhaconitine.



Reaction of Muscle-nerve from Frogs (R. temp.) *poisoned by Bikhaconitine.*—The results are fairly parallel with those obtained from indaconitine, but the average effect upon the intramuscular nervous tissue seems to be slightly weaker. Experiments *a* and *b* were performed upon brainless frogs; in *b* a vascular ligature was applied to the left leg.

	Dose per kilo.	Heart arrested.	Min. excitability.	
			N.	M.
		mins.	cm.	
a	0·002	200	44	20
b	0·012	110	Lig. 24 Open 30	23 24
c	0·024	75	45	27

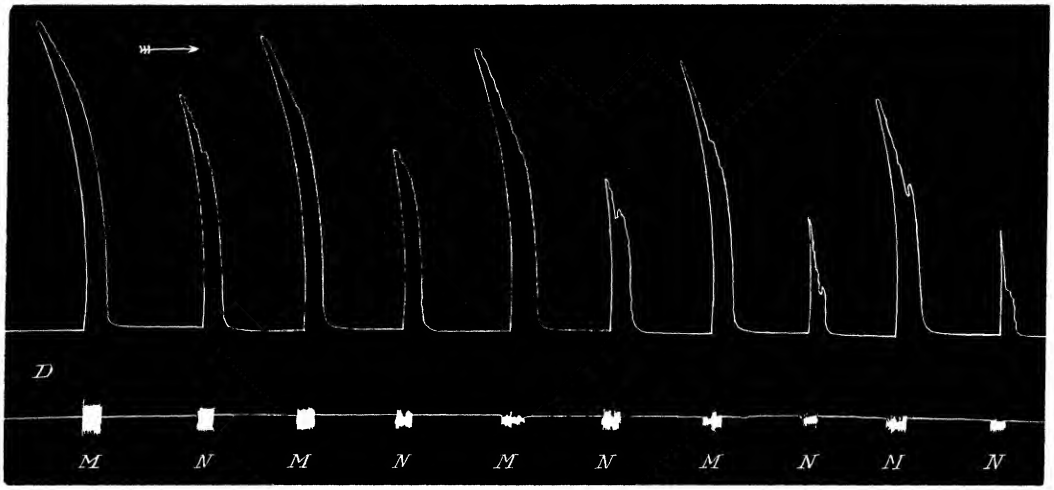
In "*b*," after a series of 10 stimulations (each 3″ faradisation) the contractions of the preparation from the open side showed a decided falling off both in altitude and in maintenance. The subsequent contractions are shown in fig. D: indirect (N) and direct (M) stimulations alternate.

c. In this experiment, though the dose was proportionately larger, less effect was produced at the periphery, owing to the earlier interference with circulation.

The effect of bikhaconitine upon nerve and muscle poisoned *in situ* appears to be slightly less (dose for dose) than is that of indaconitine.

Immersion of Nerve-muscle Preparations in Solutions of Bikhaconitine.—The results of this series of contrast experiments have been given above.

Alternating Nerve Muscle, 3 secs. faradisation. Prepared from frog poisoned by 0·012 gramme bikhaconitine per kilogramme.



Comparison of Pseudaconine from Indaconitine and from Pseudaconitine.

It has been already stated that indaconitine furnishes, as its ultimate hydrolytic product, the same base as pseudaconitine. It was thought desirable to closely compare the physiological action of the pseudaconine from both sources. The amount of material available only sufficed for observation upon frogs: in these animals the lethal dose was determined. Both alkaloids possessed a sweet taste; neither occasioned any of the aconitine effects locally when placed upon the tongue.

Action of Pseudaconine (from Pseudaconitine) in Progressive Doses upon R. temporaria.

0·4 gramme per kilogramme.—No excitement after injecting solution into dorsal sac. Reflexes rapidly disappeared; no spontaneous actions were, as a rule, attempted. In 40′ all reflex had gone and respiration had ceased, except that when placed on dorsum one slight movement of the arms and the respiratory muscles ensued. Heart 24 regular. 2 hours 47′. Reflex beginning to return. Circulation in web moderate, heart 22. Next day quite normal.

1·3 grammes per kilogramme.—As above, though action from larger dose more rapid. Pigment cells distended.

24 hours.—Reflex absent, pupils contracted, circulation moderate.

48 hours.—Sitting up but limp, cannot get off dorsum, tremulous on movement.

52 hours.—Nearly gets off dorsum.

72 hours.—Averse to movement, but hops well if roused, gets off dorsum.

1·7 grammes per kilogramme.—As above, but early symptoms are augmented and accelerated.

24 hours.—Circulation in web feeble and partial.

48 hours.—Circulation improving. Reflexes absent except faint movement of all trunk when placed on back.

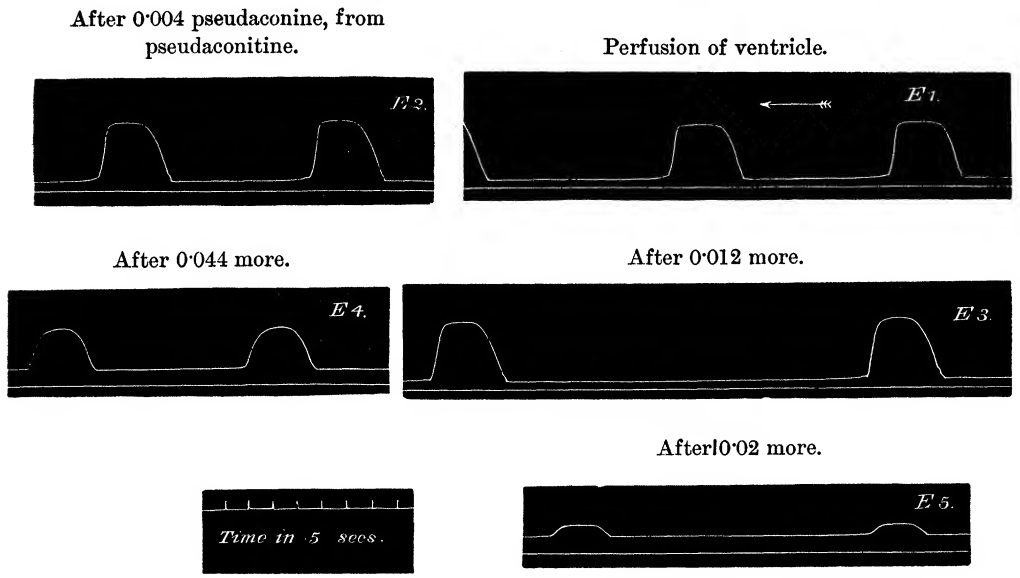
96 hours.—Sluggish eye reflex. Faint respiratory movement, gets off back.

122 hours.—Does not spring yet. Movements rather tremulous.

144 hours.—Hops fairly.

No dose of lower proportion than 1·7 per kilogramme was lethal, whereas larger doses proved so, owing to cardiac failure.

Whilst the lethal dose of this aconine is about 1·75 grammes per kilogramme, its effect in sub-lethal dose appeared to pass off more rapidly than that of pseudaconine from indaconitine. This, however, may have been due to differences in the animals under observation.



In all 0·08 gramme pseudaconine.

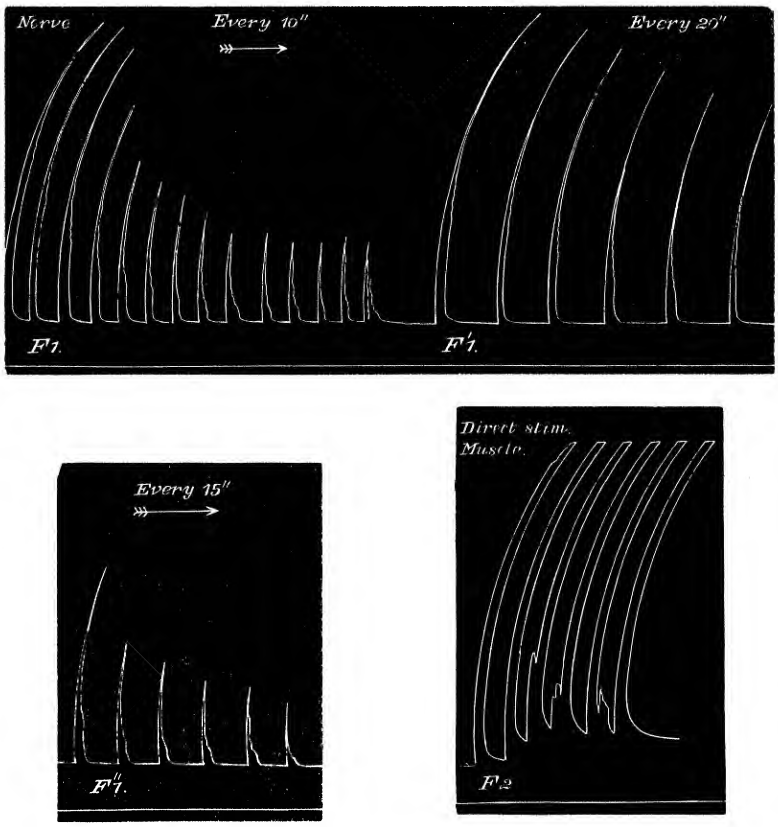
Perfusion of the Heart with Pseudaconine (from Pseudaconitine), Fig. E.—Strengthening of the ventricular systole ensues when a few cubic centimetres of solution (containing 0·02 of alkaloidal salt per 20 c.c. of menstruum) are perfused (E_2), and this effect is still apparent when 20 to 30 c.c. (E_3) have been passed slowly through the organ. When 60 to 70 c.c. have passed, a delay in the systolic phase with retarded relaxation occurs, (E_4), and beyond this amount the systole becomes distinctly feebler (E_5). A spontaneously contracting preparation beats more slowly after small and moderate doses of pseudaconine. Ringer's solution perfused through the organ, which shows evidence of the action of large doses of pseudaconine, is usually sufficient to restore the original strength of contraction.

Action on Muscle and Nerve.—When such a proportion of pseudaconine (from pseudaconitine) as 1 gramme per kilogramme is injected into the dorsal sac of a brainless frog in which one sciatic is exposed, it is found that on stimulating the peripheral end of the nerve, the contractions soon become feebler and remain altogether absent, if the stimulation is frequently repeated with but brief rest intervals. After a pause contractions are again elicited, and this phenomenon may be reproduced until under a further and increasing action of the alkaloid, all response to faradisation is eventually lost. According to the dose employed, this absence of response may last for some hours or even for a day or two. The same rapid exhaustion of excitability under stimulation, is witnessed during the earlier phases of recovery of excitability of the intramuscular motor nerves. In the uninjured animal recovering from pseudaconine, the inability to perform a series of spontaneous movements, as well as the rapid failure of the reflexes when a sufficient rest interval is not assured, is due to the same condition. The separated nerve muscle preparation gives the reaction which might be anticipated.

Experiment.—In a pegged frog (*R. temporaria*) a vascular ligature was applied to the left leg, and pseudaconine (from pseudaconitine) was injected under the skin of the abdomen in the proportion of 0·3 gramme per kilogramme. In 45′, when all reflex had disappeared from the open side, two muscle nerve preparations were made, one from either leg, and tested by faradisation. The minimal excitability on the ligatured side was 15 cm. for indirect and 9 for direct stimulation; on the open side 10 and 8 respectively.

The tracings are the result of a series of 3″ faradisations delivered every 10″ in the first series (F_1), every 20″ in the second series (F'_1), and every 15″ in the third series (F''_1). The muscle directly stimulated yields strong and well sustained contractions. This is, therefore, the result of a slight action of pseudaconine, less than one-fifth of the lethal dose having been administered.

Faradisation of nerve-muscle preparation slightly affected by 0·3 per kilogramme pseudaconine from pseudaconitine for 3 secs. on each occasion.



Action of Pseudaconine (from Indaconitine) in Progressive Doses upon R. temporaria.

0·4 gramme per kilogramme.—No excitement after injection. In 36′ all reflex and respiratory movement gone, except that, when placed on the back, one movement of the trunk and protrusion of the hyoid took place In 2 hours 40′ there is indication of return of reflexes commencing. Next day is normal.

0·8 per kilogramme.—The above symptoms accentuated. In 29 hours there is reflex response in limbs and trunk. Pupil contracted, no distinct eye reflex. In 48 hours, recovered.

1·3 per kilogramme.—In 11′ all reflex gone ; 3 hours, circulation in web is good and general.

24 hours.—No reflex, circulation good, pupil contracted.

48 hours.—Beginning to draw leg up reflexly. Partial eye reflex.

52 hours.—Legs drawn up. Cannot get off dorsum after voluntary effort, ability for further movement is temporarily suspended.

72 hours.—Lethargic, sitting up, hop short, slight tremor on movement.

1·7 per kilogramme.—As above. Circulation in 24 hours is only just moving in larger vessels of web.

48 hours.—Circulation still feeble, but more general; when placed on dorsum, a very slight movement in trunk.

96 hours.—Eye reflex doubtful, but brisk leg reflex when placed on dorsum. Circulation feeble. Respiration is *nil.*

120 hours.—Cannot get off dorsum, but moves spontaneously. Eye reflex present. Faint respiratory movements at long intervals.

144 hours.—Cannot get off dorsum, but crawls if placed on belly. Respiration stronger and more frequent.

168 hours.—Gets off back, still feeble, spring very short.

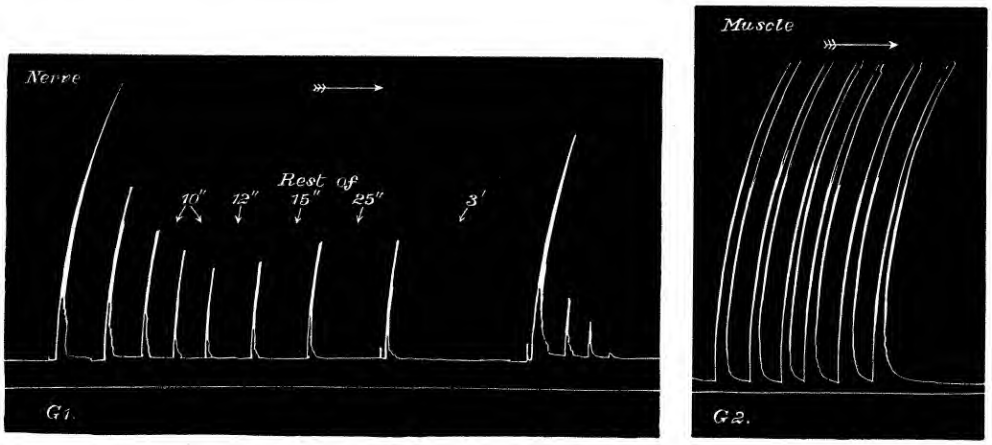
Proportions above 1·7 were lethal from failure of the heart, and so on one occasion was 1·5. It has been impossible, from scarcity of material, to make this estimation more exactly, but it is probable that the toxicity of the two pseudaconines is identical (1·75 gramme per kilogramme), although the duration of action of large doses of the indaconitine product appears to be relatively somewhat longer, a result which may be attributable rather to variations in the animals than to differences in the substances.

Perfusion of the Frog's Heart.—Solutions of pseudaconine (from indaconitine) salt (0·01 in 20 c.c. of menstruum) were found to increase the strength of the systole, and otherwise to occasion the same phenomena as those described for pseudaconine from pseudaconitine. The effect of large doses of the former seemed slightly in excess of that of the latter, but from the nature of the experiment exact contrast is difficult. The excitability of the preparation beating spontaneously so long as perfused by Ringer's solution, seems to decline on substituting pseudaconine solution, spontaneous contraction tending to become less frequent or to disappear. A good contraction is, however, elicited on stimulating.

Action on Muscle-nerve.—The same phenomena are occasioned by both pseudaconines.

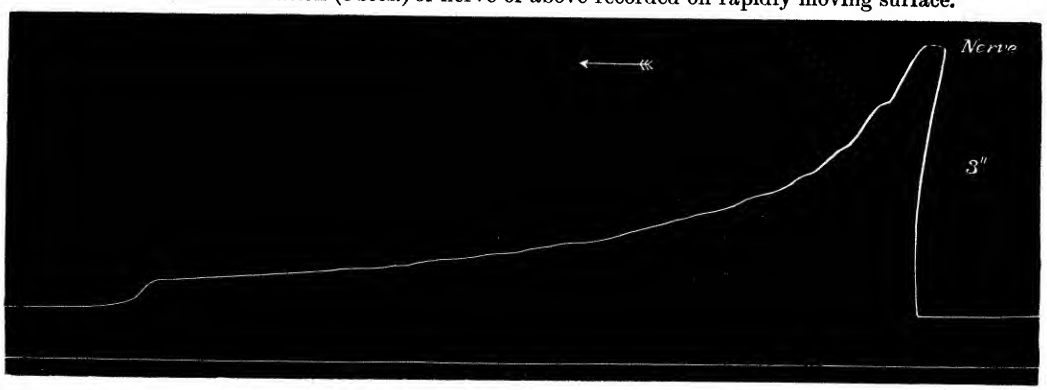
Experiment.—Pegged *R. temporaria*, vascular ligature applied to left leg. Injection 0·3 per kilogramme pseudaconine (from indaconitine). In 20′, and before reflex was quite abolished, two companion nerve-muscle preparations were made. The effect of faradisation (G_1 of nerve and G_2 of muscle) is shown in the figures. Each stimulation is of 3″ duration, and it is observable that the longer the rest interval and the greater the ensuing

G_1, faradisation of nerve-muscle preparation slightly affected by 0·3 gramme per kilogramme pseudaconine (from indaconitine) for 3 secs. on each occasion. The time-interval (marked between stimulations) varies. A pause of 3 secs. before the last group. G_2, direct stim. of muscle.



contraction, the shorter and the fainter the response. There is a rest time of 3′ before the final group is taken. A single response to 3″ faradisation recorded on a rapidly moving surface is shown (fig. H).

H. Single faradisation (3 secs.) of nerve of above recorded on rapidly moving surface.



The section on aconine in a previous paper* may stand for the pseudaconines now under discussion, in so far as the action of these upon reflex excitability of the cord, upon respiration, and upon the form of the muscle curve are concerned. All three appear to possess in common some antagonising action towards the weakening and incoordinating effect produced by the aconitines upon the frog's heart. This circumstance is referred to† when the respective action of aconitine and aconine (from *A. napellus*) was discussed.

* 'Phil. Trans.,' B, vol. 190, p. 380.
† *Ibid.*, p. 283.

Summary.

The two aconitines, indaconitine and bikhaconitine, agree in their qualitative effects with the other alkaloids of this series, aconitine, japaconitine, and pseudaconitine, which have been dealt with in our previous papers.

The toxicity of indaconitine is less than that of bikhaconitine towards warm-blooded animals; in this respect the former stands very near to the aconitine of *A. napellus*, whilst the latter being somewhat stronger than japaconitine, is to be referred to a position between this alkaloid and pseudaconitine from forms of *A. ferox* which is much the most active of the series.

The depression of the respiratory function by indaconitine is less than that produced by bikhaconitine, and to this the greater toxicity of the latter is referable. Repeated doses of alkaloids administered at regular intervals and in similar fractional proportions of the lethal dose—are followed by a more marked toxic effect when bikhaconitine is administered rather than indaconitine. Towards frogs the toxicity of the two alkaloids under discussion is practically equal, bikhaconitine is more active than indaconitine in reducing the respiratory activity. On the other hand, it is somewhat less active in abolishing the excitability of muscular and intramuscular motor nervous tissue (immersion experiments), and in reducing the ability of the muscle-nerve preparation poisoned *in situ* for the performance of work sufficient to cause fatigue. The local effect of the two aconitines when applied to the skin by inunction, is equal and similar to that of the aconitines already considered.

Indaconitine and bikhaconitine may therefore be substituted for aconitine and pseudaconitine for internal use, indaconitine being administrable in the same dose as aconitine (from *A. napellus*) and bikhaconitine in proportion of 0·75 of the unit dose of the former, whilst for local application they may be used as contituents of ointments in similar proportions to aconitine.

Pseudaconine from Pseudaconitine and Bikhaconitine.

The action of these is, towards frogs, identical. Their toxicity appears to be practically equal and their effect generally similar to that of aconine (from aconitine). Their action is in the main curari-like in character.

In conclusion it is an agreeable duty to add that many of the observations on temperature, together with control experiments upon the physiological action of the alkaloids discussed in this paper were accurately carried out by Dr. Croll, Second Assistant in the Materia Medica Department of Aberdeen University, whilst the specimens of the pure alkaloidal salts required in the experiments have been prepared in the laboratories of the Imperial Institute by Mr. A. E. Andrews, Salters' Company's Research Fellow.